Biochemistry 539M Midterm Exam
May 10, 2011

This is a take-home essay exam. Answer any three questions as completely as possible. Exam answers must be emailed to henry_levin@nih.gov before 9:00 AM on May 17. I will send an acknowledgement when I receive your exam. Late exams will lose at least one letter grade. Please start a new page for each answer and put your name in the header on every page.

1. (Gellert)
A) Combinatorial diversity of antibodies. Given the following numbers of gene segments (below), what are the numbers of possible Ig heavy chains and Ig light chains that an individual can make based on combinatorial diversity alone (ignoring junctional diversity)?

B) Assuming that any light chain can successfully pair with any heavy chain (probably slightly inaccurate), what is the number of antibodies that can be generated by combinatorial diversity?

IgH: 45 V, 23 D and 7 J
Ig **κ**: 42 V and 4 J
Ig **λ**: 28 V and 4 J

B) Junctional diversity: In combining a VH element to a DH element, the following sequences are juxtaposed: CCC from the VH side and TGG from the DJ side. But that's before nucleotides are removed or added at the junction during recombination. In these examples, we consider addition of variable numbers of nucleotides, but no deletions. Also, in this example, if no bases are added or removed, the rearrangement would be functional (the constant region would be read in-frame). For the following, added bases are represented by
"N" (can be A,T,C or G). Which rearrangement(s) would be in frame (assuming the "N's" don't create a stop codon)? Also calculate the total diversity of IgH chains, including junctional diversity, possible for rearrangements with this number of base additions.
VH: CCC **DJ**: TGG

A: CCCTGG
b: CCCNNTGG
c: CCCNNNTGG
d: CCCNNNNTGG
e: CCCNNNNNTGG
f: CCCNNNNNNTGG

2. (Hegde) A: Almost all cellular proteins are synthesized in the cytosol. However, at least half of them eventually reside in various sub-compartments (e.g., organelles), on the cell surface, or secreted. What allows the cell to decide where a given protein should be localized?

B: In most protein translocation systems, the protein to be transported across the membrane is far bigger than other molecules that should not move across the membrane. Thus, the channels for protein transport are typically quite small. How then can a large protein move through a small channel? Describe one way this problem is solved by the cell.

3. (Weinstein) The elaborate and complex pattern of the vascular networks found in all vertebrates varies from one species to another, and even from one individual to the next within a species. Although the detailed pattern of smaller vessels is variable, the basic anatomical plan of the circulatory system and patterning of larger blood vessels is extremely well conserved, even between humans and zebrafish. Recent studies have begun to unravel some of the mechanisms responsible for directing the growth of blood vessels during development.

(1) Discuss some of the possible mechanisms responsible for establishing the highly conserved pattern of major blood vessels.

(2) Discuss some of the possible mechanisms responsible for directing formation of the capillaries and smaller caliber vessels displaying a highly variable pattern.

Recent work has also highlighted examples of striking correspondence between the patterning of nerves and blood vessels

(3) Discuss some of the possible mechanisms for this correspondence.

For each one of the three points above (a) respond succinctly, limiting your answer to one paragraph, and (b) include at least one citation from the primary scientific literature (not a review article) that supports your conclusions.

4. (Dawid) "We have seen that the Wnt/β-catenin signaling pathway is critical in multiple developmental processes. Discuss and contrast early axis determination and neural crest induction in the light of experimental approaches that can be used to study the ways in which Wnt signals control these processes."

5. (Shi) What are the roles of unliganded and liganded thyroid hormone receptors during frog development and the associated transcriptional mechanisms underlying these distinct functions? Thyroid hormone is required for the development of adult epithelial stem cells in the intestine. What are the consequences of activating (ligand binding to) thyroid hormone receptor in the epithelium and non-epithelium? Discuss the likely role of non-epithelium (connective tissue) in the development of the stem cells.

6. (Ostrander) In human studies SNP chips typically contain about 1-2 million SNPs. Yet we were able to map several genes of interest using chips with just 100,000 SNPs, of which only about 60% were informative. Why? Design an experiment to test your conclusion. Take into account the specifics of you answer may be differ between breeds of dog. How would you develop parameters for a generalized answer.

7. (Lee) See next page.

8. (Misteli) Genomes are non-randomly organized in the nucleus of higher eukaryotes.
- how can you experimentally determine that a chromosome or a gene is non-randomy positioned?

- discuss known and possible molecular mechanisms by which non-random organization of genomes emerges?

- how can you experimentally test these mechanisms?

1. (Lee) You have been studying how neuron B connect to neuron Y. You used a high-power laser to kill a glial cell (G) in the path and found that B targets to X in the absence of G. However, if you kill neuron A then both X & Y disappear and neuron B randomly projects its axon. How do you interpret these results?

2. After two years of hard labor, you isolated a cool mutant (M1) that has the same phenotype as glia cell ablation. Could you design an experiment to figure out where (in which cell) this gene functions?

3. After two more years of hard work, you finally cloned the gene, N, which encodes for a novel transmembrane protein. If N is required in G, could you explain its function? If N is required in B, could you explain its function? What is your grand theory about how this circuit is constructed?